

Green Synthesis of Silver Nanoparticles Using *Nigella sativa* Callus and Cellular Suspension Cultures with Different pH Values

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ABSTRACT

The current study included an extensive protocol for the first time globally. This protocol was concerned with the green synthesis of silver nanoparticles (AgNPs) from *Nigella sativa* callus and cellular suspended cultures. The results confirmed the success of the process of green synthesis of AgNPs from *Nigella sativa* callus by the color change in the reaction solution from colorless to brown and reddish-brown as a result of the reduction of Ag ions in the extract after addition of AgNO₃ solution (1:1 v/v). UV-Vis spectroscopy was used to initially prove the existence of AgNPs, which showed an absorption peak ranging between (420-440 nm) caused by the phenomenon of Surface Plasmon Resonance (SPR) of AgNPs. (SEM) showed nanoparticle objects ranging from (18.341 - 40.932) nm for standard extracts and (17.261 - 29.13) nm for suspended cultures extracts. (EDX) proved the success of producing AgNPs in different proportions depending on the type of tissue cultures used and the difference in the pH value of the extracts (6, 9, 12), as the highest % of AgNPs production reached 4.46% in suspended cultures extracts from pH 12, followed by callus extracts, which reached 4.12% at pH 12. biologically prepared AgNPs proved their effectiveness in inhibiting the growth of Gram⁺ and Gram⁻ bacteria, as the inhibition zone ranged (19– 35 mm) depending on the type of extract and the degree of acidity (pH) applied to the extract.

Keywords: *Nigella sativa*, silver nanoparticles, callus, suspension culture.

INTRODUCTION

Nigella sativa L. is an annular herbaceous plant belonging to the ranunculaceae family, of high medicinal value, widely used in ancient times in folk medicine, and is known as a miraculous herb due to its wide range of medicinal and pharmacological properties (Yimer *et al.* 2019) for containing biologically active compounds showing broad therapeutic efficacy (Jin, 2019; Mechraoui *et al.*, 2018).

Plant tissue culture is an important tool for the continuous production of active compounds, including secondary metabolites and engineered molecules, and for plants propagation using nutrient media, under sterile conditions without dependence on a specific geographical location or season (Espinosa-Leal *et al.*, 2018). cellular suspensions culture has become one of the best tissue culture techniques in the production of effective metabolic compounds from callus cultures due to its lack of dependence on the natural environment and its short production cycle (Arya *et al.*, 2020).

Nanotechnology aims to deal with ultra-small particles whose dimensions do not exceed 100 nm, called nanoparticles, and there are many distinct technologies by which silver nanoparticles can be synthesized, including "physical" and "chemical", which are effective technologies but cause damage and side effects on the environment and human health, in order to use flammable substances and harmful radiation, as well as limit their applied Value. Hence the need to find a better technology, which is "biological" for the synthesis of nanoparticles from bacteria, algae and plants, it is economical, inexpensive, energy-saving, being environmentally friendly, producing less waste and safer for health (Mohamed *et al.*, 2019; Chand *et al.*, 2020), and in particular vegetative manufacturing using plant extracts, which has outperformed other technologies by containing a wide range of biomolecules that act as reducing and stabilizing agents (Capping agent) for nanoparticles and thereby improving their production (Roy and Das, 2015).

It is known that there are many different microorganisms such as bacteria, mold, fungi, etc., that cause infection to humans and other organisms. To combat these pathogens, many antimicrobial compounds and antibiotics have been discovered. But, slowly and over time, microorganisms began to develop their resistance against most antibiotics, causing the problem of treating these diseases (Komolafe, 2003), researchers focused on alternative treatments or methods that can overcome antibiotic resistance and at a relatively low cost.

Nanoparticles have become a good alternative (Kim *et al.*, 2011) and among the most important and widely used nanoparticles are silver nanoparticles (AgNPs) for their broad biological activity against microbes, low cytotoxicity and can be used in a variety of activities *in vitro* and *in vivo* (Botcha and Prattipati, 2020) accordingly, over the past decade, some medicinal plants have been used in the synthesis of nanoparticles, including *Nigella sativa*, where researchers have succeeded in synthesizing silver and gold nanoparticles from aqueous extracts of seeds and leaves (Veeramani *et al.*, 2020; Anwar *et al.*, 2021; Amooaghaie *et al.*, 2015), but not from callus tissue , The current study aimed to synthesize silver nanoparticles for the first time globally from callus cultures (standard and cellular suspension cultures) of the *Nigella sativa* plant, and then to study their effectiveness in inhibiting against bacterial growth.

MATERIAL AND METHODS

The practical steps for manufacturing and diagnosing silver nanoparticles AgNPs were carried out in detailed steps:

Preparation of plant material (biomass):

- The production of *Nigella sativa* callus culture:

The seeds were cultured on MS (Murashige and Skoog, 1962) medium after surface sterilization by submerge seeds in 96% ethanol for 2 minutes with constant stirring, then under sterile condition, seed were transferred to sodium hypochlorite diluted with distilled water (1:2 v/v) respectively,

after 4 minutes washed them with sterile distilled water 5-7 times and dried on filter paper. Then incubated at a temperature of $22\pm 2^{\circ}\text{C}$ under dark conditions for seed germination and seedling growth. For callus initiation, the seedlings were taken at the age of 21 days and were cut into (2 stems + 1 node), and planted on MS medium supported by 2,4-D (10^{-6} M) (Albaker, 2002). Subculture the callus cultures continued at regular intervals of 25 to 30 days on the same medium.

- Production of cellular suspensions culture:

Cellular suspensions culture was prepared based on the method (Al-Saleh and Al-Naimi, 2010) with some modification, by taking 1.5 g of fragile callus tissue for each sample and placing it in flasks containing 20ml of liquid MS medium supported by 10^{-6} M of 2,4-D, then placing it in the Rotary incubator (Shaker incubator, Burn Swich, USA) at 100 r/minute with temperature of $25\pm 2^{\circ}\text{C}$ and 8/16 dark/ light duration for 21 days, re-culturing was done every week. After that, the cells were collected by filtering the suspension using filter paper (Whatman No. 1) then washed and filtered, 3 times using sterile distilled water to use it directly in the next step.

Preparation of *Nigella sativa* aqueous callus extract:

Depending on the modified (Aref and Salem, 2020) method, took 10 g fresh weight of 60 days old callus samples and prepared their extracts separately and in the same way, first the sample was washed with sterile deionized water, crushed in a ceramic mortar, placed in a glass beaker and completed the volume to 100 ml of sterile deionized water, then heated the mixture up to 70°C for 3 hours with constant stirring on a magnetic stirrer, after which the mixture was left to cool, for ultrasonic treatment for 5 minutes, and finally the extract was filtered using Whatman no.1 filter paper. After cooling the aqueous callus extracts were obtained.

Change the pH of the aqueous extract:

The pH of the extracts prepared in the previous step is fixed at the values (6 - 9 - 12) by adding drops of sodium hydroxide (NaOH).

Preparation of silver nitrate solution (AgNO_3):

weighing 0.17 g of silver nitrate and dissolve it in 1 liter of sterile non-ionic water, mix well and keep it in dark bottles to prevent it from being affected by light until use.

Production of silver nanoparticles AgNPs:

On the magnetic stirrer, an aqueous callus extract with various degrees of acidity was added drop by drop to a solution of silver nitrate (1:1 v/v). Then put the mixture in a water bath at a temperature of 40°C for 3 minutes.

Detection of silver nanoparticles Ag-NPs formation:

- visible color change:

- UV-Vis Spectrophotometer:

One of the most widely used techniques for characterizing biologically produced Ag nanoparticles is ultraviolet visible spectroscopy, which determines the optical density. The absorbency of silver nanoparticles AgNPs was measured with a wavelength ranging from 200 to 800 nm. three minutes after the color change.

- Scanning Electron Microscopy-Energy Dispersive X-ray (SEM-EDX)

The SEM-EDX tests were conducted in the laboratories of the Department of Physics/ Collage of Science/ University of Basra. Microscopic images of the shape and size (average grain diameter) of silver nanoparticles were taken using a scanning electron microscope with high - resolution imaging-the resolution of low voltage [1 kV] is 1.8 nm in low vacuum mode and 1.4 nm in high vacuum mode, and connected with the reflection of energy-scattering X-rays (EDX) which give the percentage of AgNPs that formed in the solution. First, the sample is subjected to ultrasound for 15

minutes. This solution was dropped on to carbon-coated copper grids, and left to evaporate, leaving a thin film on the substrate.

Antibacterial activity of silver nanoparticles:

The antibacterial activity of silver nanoparticles was tested on two types of Grams and Gram positive bacteria (*Escherichia coli* and *Staphylococcus aureus*). Using the disk diffusion method (Narms, 2002) in a petri dish, spread 1 ml of bacterial suspension uniformly on solid growth media Muller Hinton (M.H). Aqueous extracts of callus and cellular suspensions, with different pH values individually were Impregnated in sterile paper tablets (6 mm diameter), dried and then placed on the surface of the solid medium in the dishes. The dishes incubated for 24 hours at a temperature of 37°C. The inhibition zone was then measured to determine the effectiveness of the AgNPs against the bacterial growth.

RESULTS AND DISCUSSION

Production of *Nigella sativa* callus culture:

The process of callus production was carried out by cultured the seeds on MS0 medium, after the seedling's growth, they were cut and cultured on MS media supported with (10^{-6} M) 2,4-D. The callus tissue is distinguished by a bright green color and a fragile texture, as shown in Fig. (1):

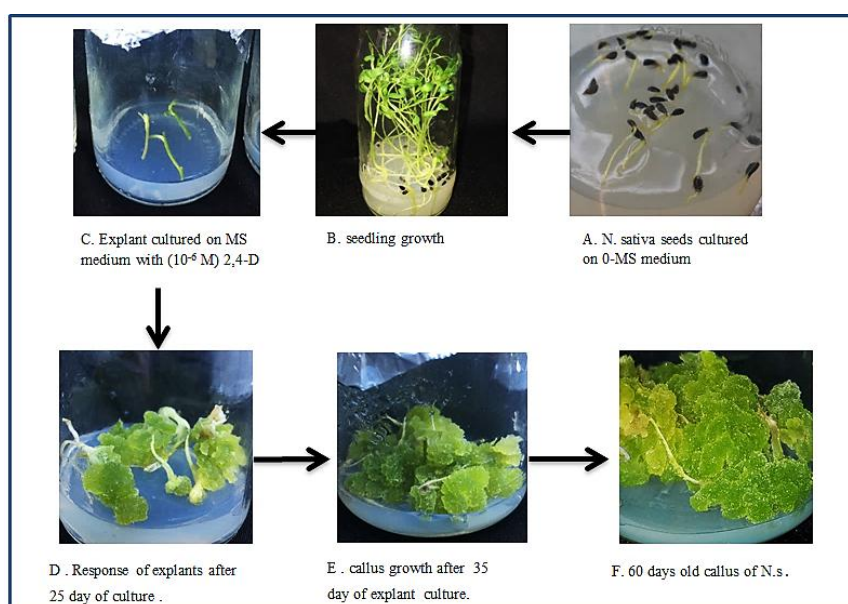


Fig. 1: A diagram showing the stages of *Nigella sativa* callus initiation and its culture production.

Detection of silver nanoparticles AgNPs synthesis from *Nigella sativa* Callus:

The composition of silver nanoparticles is observed by several indicators and as follows:

- Visible color change:

The results of the current study showed the possibility of biosynthesis silver nanoparticles AgNPs using *Nigella sativa* callus cultures as shown in Fig. (2) by observing the color change of the filtrate extract added to the silver nitrate solution from colorless to yellow, orange or brown depending on the type of culture used to prepare the extract and the pH value. The results of the color change proved that there is a difference in the intensity of the color depending on the type of callus used in the preparation of the extract and the pH value of the extract.

The results showed that when the callus extract was added to the silver nitrate solution, the color of the solution changed from colorless Fig. (2) to pale yellow if the pH value of the solution was 6, if the pH value of the solution was 9, the color changed to greenish brown, and at pH 12, the color

change was observed to reddish brown Fig. (3), and if the extracts of cellular suspensions were added to the silver nitrate solution, the color also changed to varying degrees depending on the installed pH value, from colorless to orange if the pH value of the solution was 6, and if the pH value of the solution was 9, the color changed to greenish brown, and at pH 12, the color change was observed to reddish brown Fig. (4). This change is caused by the phenomenon of surface plasmon resonance, which confirmed the formation of silver nanoparticles in the reaction mixture, and the period of color change of the solution decreased with an increase in the pH value, which is evidence that the pH affects the reaction speed significantly.

The resulting color change in the current study is similar to that found by (Almatroudi *et al.*, 2020) in his experiment to manufacture silver nanoparticles from *Nigella sativa* seed extract, the color of the solution changed from pale yellow to reddish brown compared to the control treatment that preserved the color of the extract.

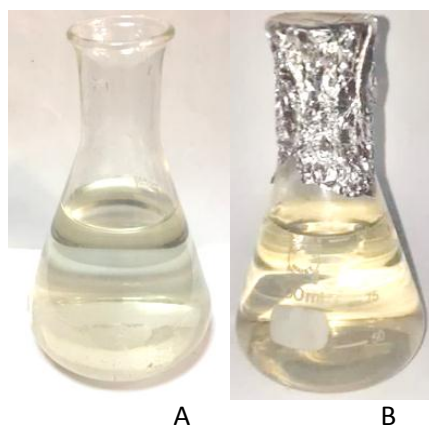


Fig. 2: color of: A- aqueous callus extract. B- silver nitrate solution of 1 mM

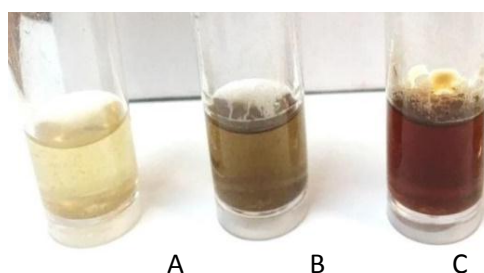


Fig. 3: The color change of standard callus extracts after adding them to the silver nitrate solution. And after three minutes in the water bath at 40 C°

A-pale yellow color at pH = 6
B-greenish-brown color at pH= 9
C - reddish brown color at pH= 12

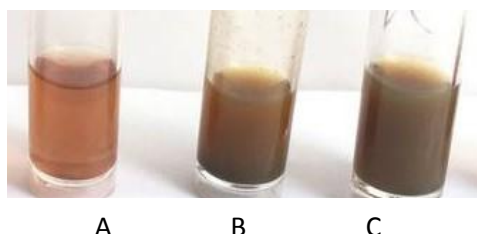


Fig. 4: The color change of cellular suspensions extracts after adding them to the silver nitrate solution. And after three minutes in the water bath at 40 C°

A-orange color at pH = 6
B-brown color at pH= 9
C-dark brown color at pH= 12

Ultraviolet and visible (UV-Vis) Spectrophotometer:

The results of the current study have shown the possibility of silver nanoparticles synthesis from *Nigella sativa* callus by knowing the absorbency of ultraviolet and visible rays within the range (200-800 nm) of the solution prepared by adding the aqueous extract of various callus cultures to the silver nitrate solution, and it is one of the important techniques for detecting nano compositions as a result of irritation and vibrations in the plasmon (electron or gap) at the metal surface.

Fig. (5) shows the absorption spectrum of the aqueous extract used in this study was performed at pH 6, 9 and 12 and after adding them to a solution of silver nitrate and introducing them into a water bath at a temperature of 40C° for three minutes, it was noted that the solution Silver nitrate AgNO_3 alone, as well as the callus aqueous extracts alone, did not show any absorption peak indicative of the absence of silver nanoparticles in them, on the contrary, when mixing aqueous extracts with silver nitrate, which reduced negative silver ions Ag^- to metallic silver particles Ag^0 , the absorption peak appeared clearly at a wavelength ranging between (420-440 nm).

The degree of acidity of the solution played a role in the difference of the absorption peak, reaching (440 nm) at pH 6 and PH 9, and (420 nm) with the increase in pH to 12 for silver nanoparticles prepared from the standard aqueous extract, Fig. (5 - A). Considering the absorption peaks of silver nanoparticles formed from extracts of cell suspension cultures, a small variation in the intensity of absorption was observed depending on the degree of acidity, and the absorption peaks were at pH 6 (420 nm) and (440 nm) at pH 9 and PH 12 Fig. (5 - B).

Studies show that almost identical peaks were obtained by the researcher (Rohini *et al.*, 2019), which confirmed that the UV-Vis peaks of silver nanoparticles biologically-prepared by aqueous extract of *Nigella sativa* seeds appeared at Wavelength (390 nm), as shown (Almatroudi *et al.*, 2020) that absorption peaks of silver nanoparticles prepared from *Nigella sativa* seeds appeared at Wavelength (400 nm), and the silver nanoparticles from callus cultures of *Solanum incanum* had an absorption peak at (440) nm (Lashin *et al.*, 2021).

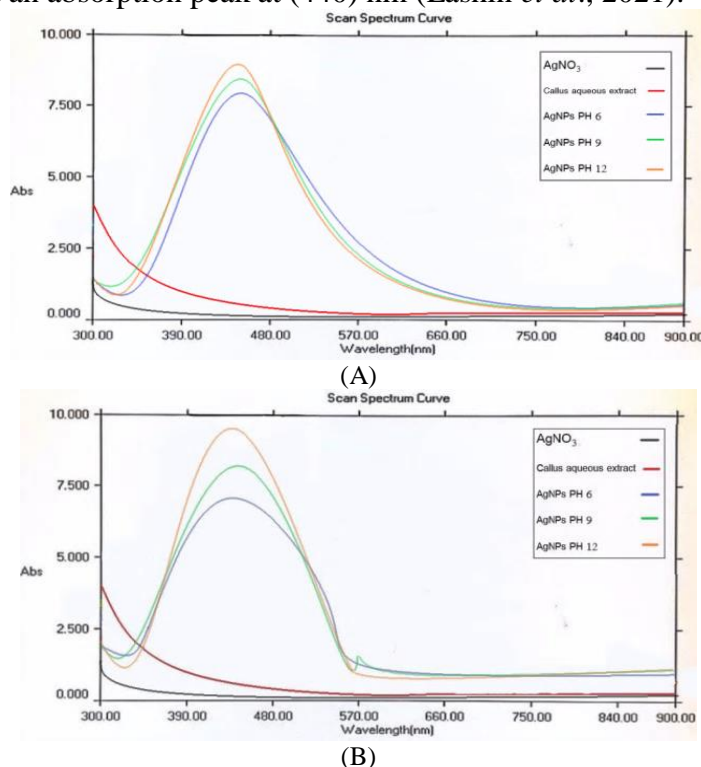


Fig. 5: UV-Vis spectrum of silver nanoparticles AgNPs synthesized from aqueous extracts of *Nigella sativa* callus cultures with different pH value after adding them to a silver nitrate solution

A - silver nanoparticles synthesized from standard callus culture.

B-silver nanoparticles synthesized from cellular suspensions cultures.

Scanning Electron Microscopy (SEM)

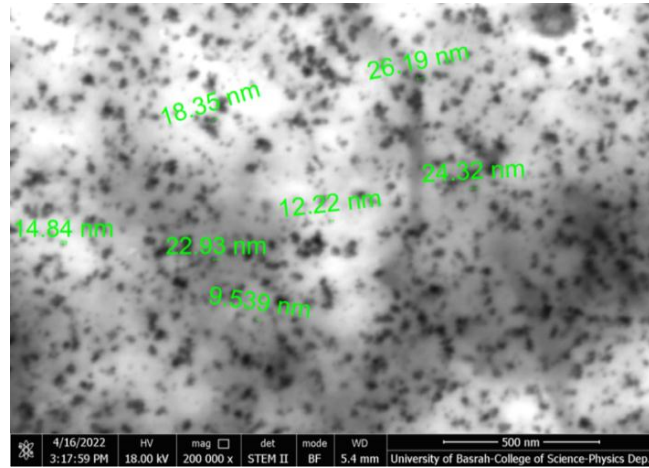
By measuring the scanning electron microscope, it is possible to determine the shape and size of nanoparticles and their distribution with high accuracy and magnification power. scanning electron microscope images of silver nanoparticles prepared using callus cultures of the *Nigella sativa* plant show the distribution of silver atoms that are in the form of spherical grains with an average grain size (17.261 nm – 40.932 nm) and these grains are homogeneous and non-contiguous.

The results obtained and confirmed in (Table 1) showed that the size of silver nanoparticles prepared from standard *Nigella sativa* callus with pH 6 ranged from (9.539nm - 26.19nm) at an average of 18.341 nm, and from (14.35nm - 32.37nm) at pH 9 to have an average particle size of 23.988 nm, and an average of 40.932 nm for particle size when increasing PH up to 12 in which the particle size ranged from (33.73nm – 59.44nm), Fig. (4).

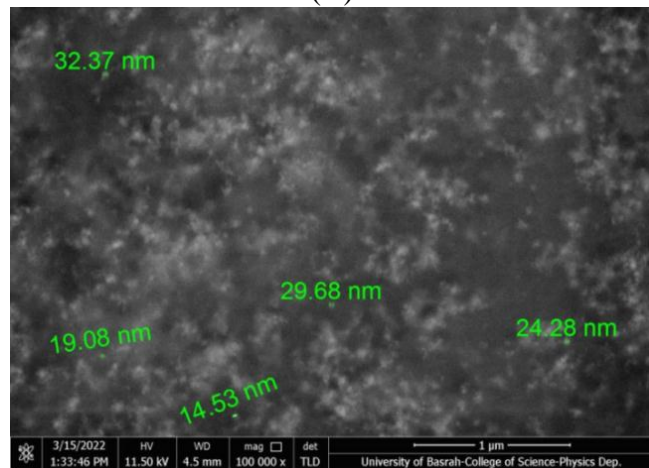
The extracts of cellular suspensions recorded a noticeable superiority over the standard extract in terms of the small size of the granules formed, the size of silver nanoparticles formed in them ranged from (4.151nm - 37.12nm) and the average size of 17.261 nm at pH 6, and the average size of silver nanoparticles increased to 24.59 nm at pH 9, as the particle size had (16.41nm – 30.16nm). The average size of the prepared silver nanoparticles was 29.13 nm at pH 12 and the size of the formed particles ranged from (4.151nm - 47.32nm), Fig. (5).

The results of scanning electron microscopy of the grain average size obtained in this study exceeded the results obtained by (Almatroudi *et al.*, 2020) the size of silver nanoparticles obtained from *Nigella sativa* seed extract, which reached (100-150) nm, on the other hand, the study was significantly consistent with the results (Lashin *et al.*, 2021), which used the *Solanum incanum* in the production of silver nanoparticles and proved their existence in sizes ranging from (15 to 60) nm with an average \approx 31.1 nm.

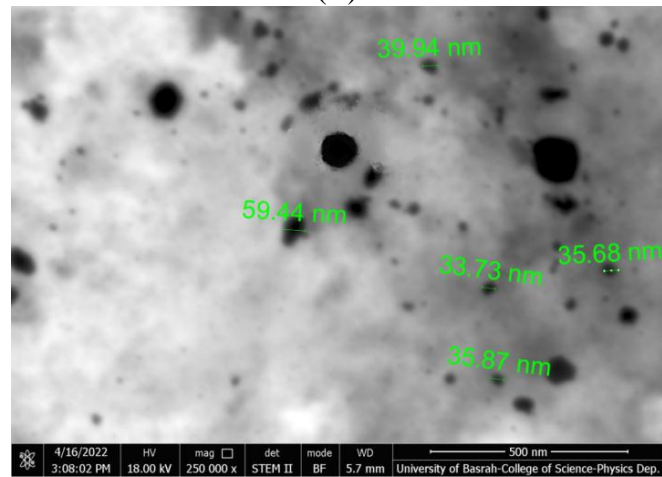
One of the studies supporting the effect of PH on the size of green synthesized nanoparticles from the same plant (Boudiaf *et al.*, 2021) confirmed an increase in the particle size of nickel oxide nanoparticles NiO prepared from *Nigella sativa* seed extracts by increasing the solution pH from 7 to 9 and 11



(A)

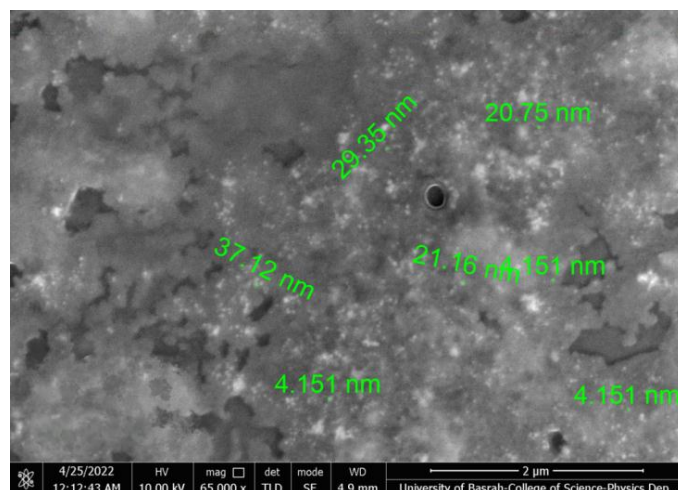


(B)

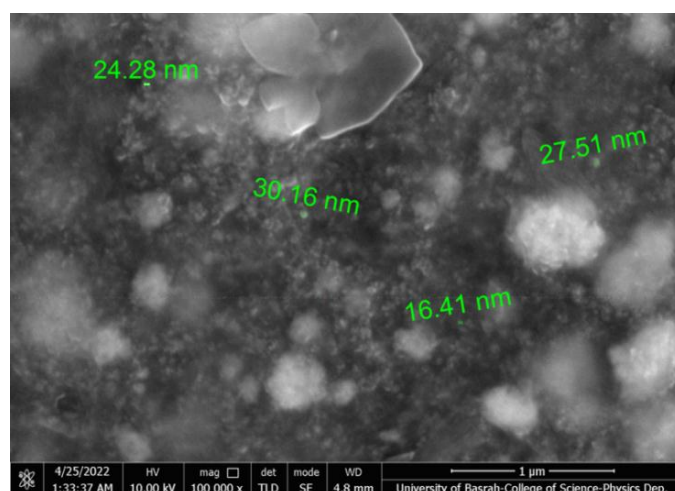


(C)

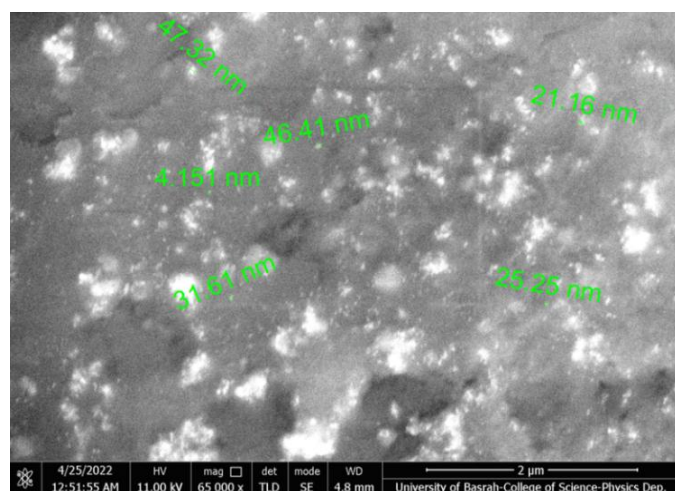
**Fig. 6: By SEM the spherical shape and size of the green synthesized AgNPs from standard *Nigella sativa* callus extract of different pH:
A-pH 6 B-pH 9 C-pH 12**



(A)



(B)



(C)

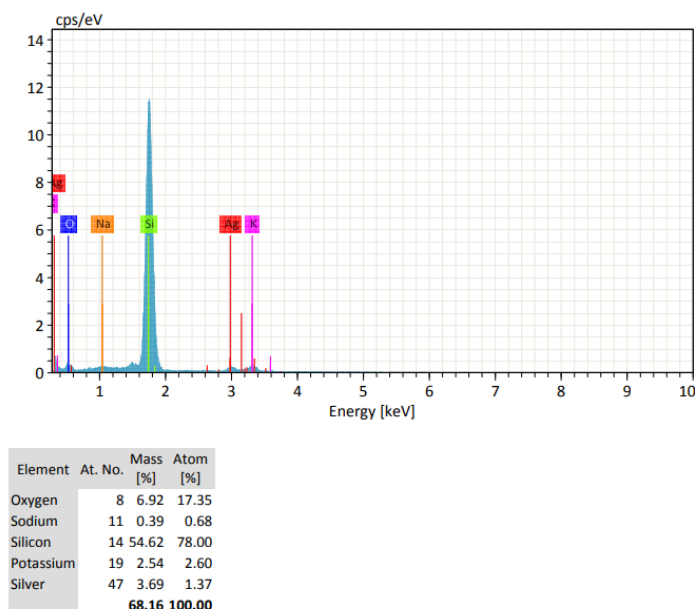
Fig. 7: By SEM the spherical shape and size of the green synthesised AgNPs from *Nigella sativa* cellular suspended callus extract of different pH:
A-pH 6 B-pH 9 C-pH 12

Table 1: average of silver nanoparticles size prepared from *Nigella sativa* callus extracts in different pH values.

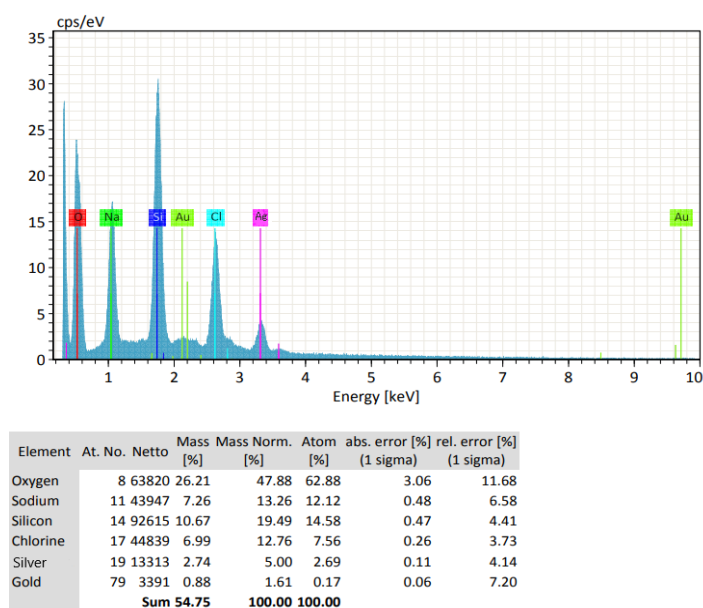
Extract sample	Average particle size (nm)		
	pH 6	pH 9	pH 12
St	18.341	23.988	40.932
CSC	17.261	24.59	29.13

Energy Dispersed X-ray spectroscopy (EDX):

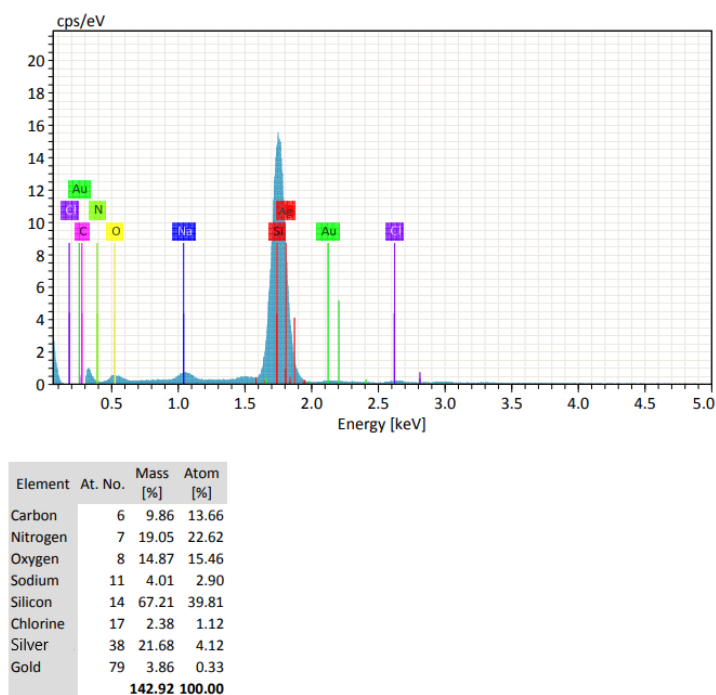
The qualitative and quantitative initial components of silver nanoparticles synthesized from callus extract were measured by EDX analysis. From the (Table 2), the EDX Spectra confirmed the presence of AgNPs in the samples (1.37% - 4.46%), as the percentage of AgNPs synthesized from standard callus extracts was (1.37%, 2.69%, 4.12%) at pH (6, 9, 12) respectively, Fig. (6). While the percentage of silver nanoparticles formed from cellular suspension of callus cultures was increased (1.5%, 2.73%, 4.46 %) at the pH value (6, 9, 12) respectively, Fig. (7). The results of the EDX analysis showed the presence of other elements such as oxygen O, carbon C, nitrogen N, sodium Na, potassium K and others in varying proportions, this may be due to the dispersion of metabolic compounds in the callus that coated silver nanoparticles such as proteins, carbohydrates and amino acids by X-ray emissions (Khan *et al.*, 2016).



(A)



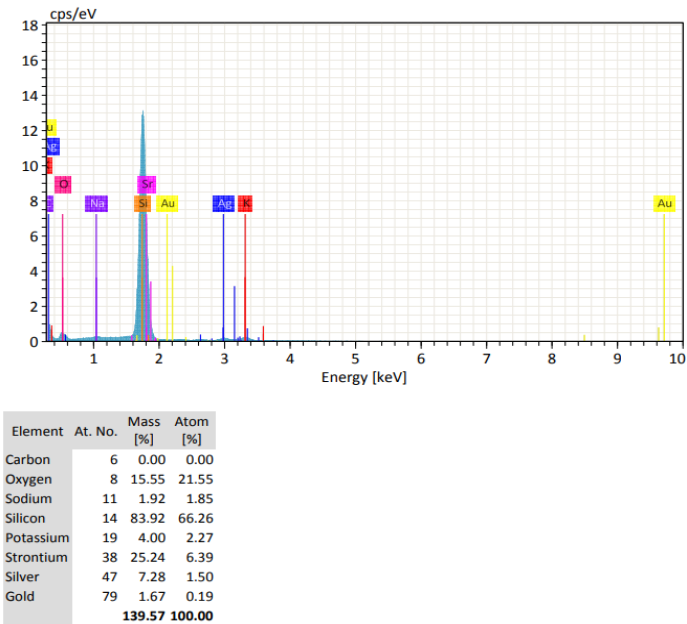
(B)



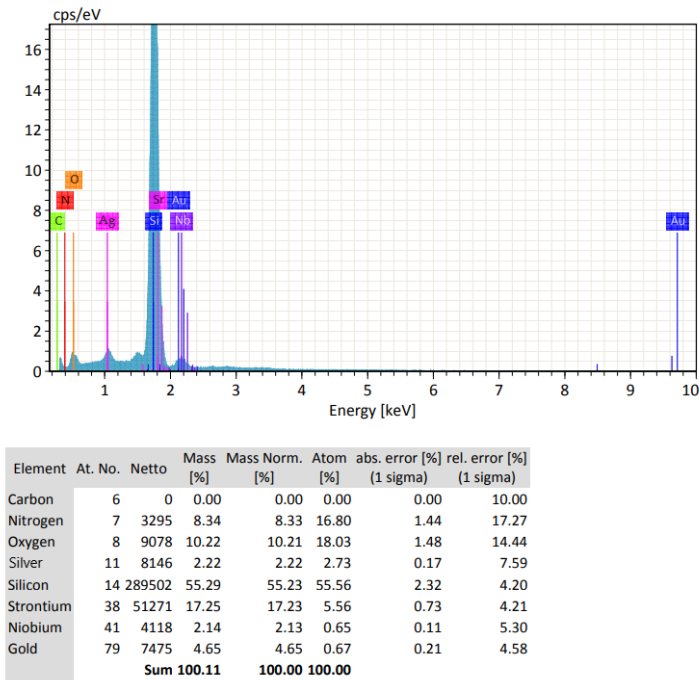
(C)

Fig. 8: percentage of silver nanoparticles synthesized from *Nigella sativa* standard callus extracts at different pH value.

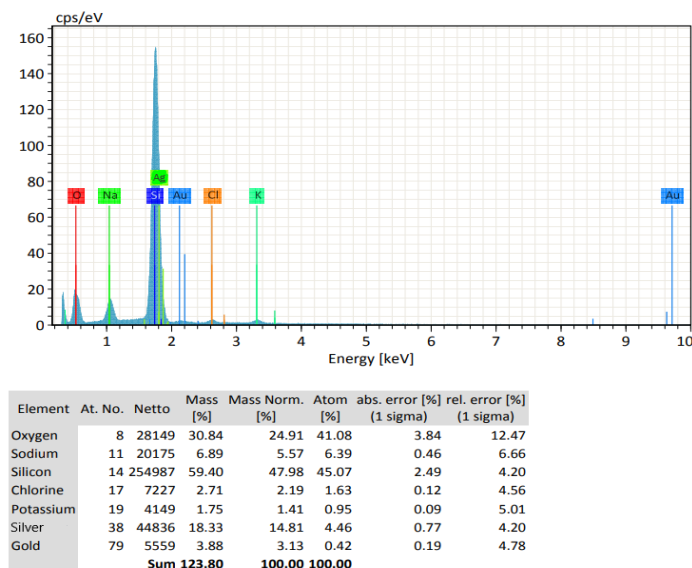
A-at pH 6 B - at pH 9 C-at pH 12



(A)



(B)



(C)

Fig. 9: percentage of silver nanoparticles manufactured from *Nigella sativa* cellular suspensions extracts with different pH value.

A-at pH 6 B - at pH 9 C-at pH 12

Table 2: Silver nanoparticles percentage synthesized from *Nigella sativa* callus cultures extracts with the variation of pH value, by EDX analysis

Extract sample	Silver nanoparticles percentage %		
	pH 6	pH 9	pH 12
St	1.37	2.69	4.12
CSC	1.5	2.73	4.46

It is obvious from the results, there is a variation in the size and percentage of silver nanoparticles formed by different type of extracts, as the cellular suspensions extracts exceeded the standard callus extracts, as they recorded a higher percentage and a lower size with different pH value for the extracts, and this may be due to an increase in the bioavailability and improved optimal conditions for the production of secondary metabolic compounds and other important compounds in cell suspension cultures (Scholz *et al.*, 2009), characterized by a homogeneous nature in terms of the distribution of suspended cells and an increase in their surface area, which in one way or another leads to optimal exploitation of nutrients in the medium and an increase in complex physiological activities, and consequently, a high rate growth, reproduction, synthesis and accumulation of biologically active compound (Moscatiello *et al.*, 2013) in larger quantities, more efficiently and with a shorter production cycle than standard callus cultures and other culture (Mathew and Sankar, 2011, 2014), as the productivity of biomass and its compounds for cellular suspensions can be more than a gram of dry biomass per liter of medium per day (Babich *et al.*, 2020), the quantity and quality of nanoparticles is greatly influenced by active plant compounds that act as capping agent covering and reducing agents (such as terpenes, flavonoids, carbohydrates and others) and so on increasing their abundance in cellular suspensions extracts, improves the quantity and quality of green synthesized silver nanoparticles, silver nanoparticles tend to agglomerate naturally due to their high surface energy, and with the presence of metabolic and active compounds, they spontaneously bind to the surface of silver nanoparticles (Malik *et al.*, 2014; Hebeish *et al.*, 2016; Tanner *et al.*, 2015; Ahmed *et al.*, 2016) and thus limit the increase in the size of nanoparticles, increase their concentration, control their stability and shape.

It should also be noted that the size and amount of silver nanoparticles increased with the rise of the pH of different *Nigella sativa* callus cultures extracts. This may be due to the influence of the pH value on the activity of phytochemical compounds contained in plant extracts, the formation of nuclear centers, and the reduction rate of the AgNO_3 metal salt significantly leading to an increase in the reaction speed, which lead to the high particle size and concentration (Seifipour *et al.*, 2020 ; Marciniak, 2020), while the acidic conditions of the medium at pH 3 did not observe the formation of any of these particles, which is evidence that nanoparticles formation under strongly acidic conditions is not possible (Hong *et al.*, 2022).

Antibacterial activity of silver nanoparticles:

The effectiveness of biologically prepared silver nanoparticles against two types of Gram positive and negative bacteria (*Escherichia coli* and *staphylococcus. aureus*).

The data obtained in (Table 3) showed that silver nanoparticles manufactured by *Nigella sativa* callus extract had a notable effect on bacterial growth, by measuring the diameter of the inhibition zone of all samples and comparing it with the inhibition zone of the antibiotic cefotaxime (CTX 30) of (10 mm), the diameter of the inhibition zone of silver nanoparticles prepared from standard callus (St) and from cultures of cellular suspensions callus culture (CSC) with different pH, where from 19 mm to 35 mm, and experimentally the sensitivity of bacteria to aqueous extracts alone and silver nitrate solution alone showed the bacteria resistance and not significantly affected, while silver nanoparticles prepared from Standard callus showed of its effectiveness in inhibiting the growth of *S.aureus* and *E.coli* bacteria ,as follows: the inhibition diameter (30 mm and 30 mm) at pH 6, (25 mm and 27 mm) at pH 9 and (19 mm and 23 mm) at pH 12, Fig. (8 - a). Silver nanoparticles prepared from cellular suspension callus culture extracts had a higher inhibitory capacity with a diameter of (35 mm and 30 mm) with pH 6, (30 mm and 28 mm) pH9 and (15 mm and 18 mm) pH 12, Fig. (8 – B). And here a decrease in antibacterial effectiveness can be observed with an increase in the pH value.

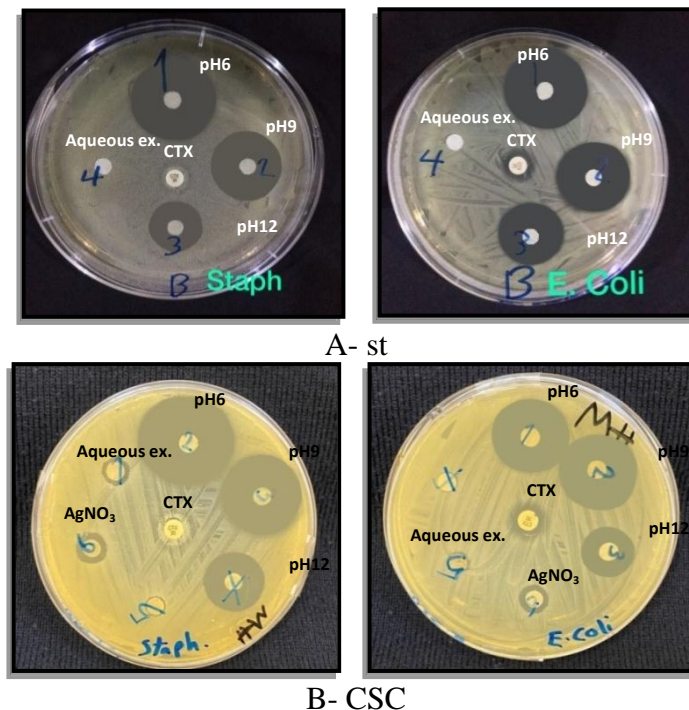


Fig. 10: Inhibitory effect of silver nanoparticles on *E. coli* and *staph. aureus* bacteria that growing on M.H solid media.

A-The inhibitory effect of AgNPs prepared from the aqueous extract of the standard *Nigella sativa* callus with different pH.

B-The inhibitory effect of AgNPs prepared from the aqueous extract of *Nigella sativa* cellular suspensions callus cultures with different pH.

Table 3: Inhibitory activity of silver nanoparticles biologically prepared from *Nigella sativa* callus extracts

Extract sample		inhibition zone (mm)	
		<i>Staph. aureus</i>	<i>E. coli</i>
St	CTX	10	10
	aqueous extract Callus	0	0
	AgNPs , pH 6	30	30
	AgNPs , pH 9	25	27
	AgNPs , pH 12	19	23
CSC	CTX	10	10
	aqueous extract Callus	1	1
	AgNO ₃	2	2
	AgNPs , pH 6	35	30
	AgNPs , pH 9	30	28
	AgNPs , pH 12	20	23

Silver nanoparticles show the advantage of their antimicrobial efficacy compared to other nanoparticles due to their extremely large surface area, providing an improved reaction on microorganisms (Logeswari *et al.*, 2015). Many factors influence the bacterial sensitivity to silver nanoparticles, the first of which is the concentration of bacterial growth, bacterial chains and components of the growth medium (Anigol *et al.*, 2017). As well as the various physical and chemical properties of silver nanoparticles include size, shape, concentration, crystallization, surface charge and colloidal state (Brussel *et al.*, 2018).

In turn, these physical and chemical properties mostly depend on the method used to synthesize nanoparticles. It is likely that the effectiveness of silver nanoparticles against microorganisms is due to the silver ions released by them or the silver nanoparticles themselves, as they have a special affinity towards certain groups such as sulfhydryl, which is present in the enzymes and proteins of the cell membrane, and by their association the effectiveness of these proteins and enzymes is disrupted, inhibit formation of ATP energy compound, inhibition of respiratory enzymes, generation of reactive oxygen species ROS. Thus, many important cellular processes such as protein synthesis, protein multiplication and cell proliferation are disrupted, and then the death of the G⁻ and G⁺ bacterial cell (Yan *et al.*, 2018; Xu *et al.*, 2020; Dawadi *et al.*, 2021).

As well as electro kinetic attractions between the positive charge of silver nanoparticles and the negative charge of the bacterial cell surface, lead to some changes such as cytoplasmic contraction, membrane detachment and, ultimately, rupture of the cell membrane (Boateng and Catanzano., 2020).

The effectiveness of silver nanoparticles against *Escherichia. coli* and *staphylococcus. aureus* in the current study has been experimentally proven in several recent studies (Rizwana *et al.*, 2022; Attallah *et al.*, 2022; Bhat *et al.*, 2022).

The results of the current study were consistent with (Anigol *et al.*, 2017) found that increasing the pH of silver nanoparticles reduced their antibacterial efficacy. This may be due to the size of silver nanoparticles, which increases with the pH value due to an increase in the reaction speed, according to a study (Marciniak, 2020), where the smallest size of silver nanoparticles was obtained at PH 6 and the particle size increased at pH more than 6.

As the size of nanoparticles increases, their inhibitory effects decrease (Kalia *et al.*, 2020). In addition, the lower the concentration of silver particles, the lower the inhibitory effectiveness of bacterial growth (Lashin *et al.*, 2021).

CONCLUSIONS

The search for a variety of different, environmentally friendly and inexpensive sources of green manufacturing nanoparticles has become one of the main challenges of Biotechnology. More recently, callus tissue and its metabolic compounds have received more attention as a green approach to the synthesis of nanoparticles.

This study succeeded for the first time in synthesizing silver nanoparticles AgNPs from *Nigella sativa* callus cultures, which played a dual role as a covering and fixing agent for the formation of AgNPs. Based on UV-vis and SEM-EDX spectroscopy, which confirmed the formation of spherical Ag-NPs in varying sizes and proportions, it can be said that the callus cultures and cellular suspension cultures are effective in the preparation of silver nanoparticles with different pH values of their extracts. The pH of the aqueous extract effect on the reaction speed and hence on the size and concentration of the silver nanoparticles formed.

The formed silver nanoparticles also showed antimicrobial activity against pathogenic bacteria including *Escherichia. coli* and *staphylococcus. aureus*. The extracts of cellular suspension cultures significantly outperformed the standard extract in the production of silver nanoparticles with a higher concentration and a smaller size to acquire a high inhibitory ability for bacterial growth. Ultimately, this study opens up new possibilities for plant tissue culture technology in general and cellular suspension culture in particular can be exploited in the manufacture of various nanomaterials locally, and the use of these nanoparticles in all scientific and applied fields.

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التصنيع الخضري لجسيمات الفضة النانوية باستخدام مزارع الكالس والمعلقات الخلوية لنبات حبة البركة *Nigella sativa* باختلاف قيم درجة الحمضية pH

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الملخص

أولت الدراسة الحالية الاهتمام بإنتاج جسيمات الفضة النانوية (AgNPs) Silver nanoparticles خضرياً ولأول مرة عالمياً من مزارع الكالس ومزارع المعلقات الخلوية لنبات حبة البركة *Nigella sativa*، وبيان تأثير اختلاف درجة الحمضية pH للمستخلصات على عملية توليف جسيمات الفضة النانوية. أكدت النتائج نجاح عملية إنتاج جسيمات الفضة النانوية AgNPs مبدئياً من خلال تغير اللون في محلول التفاعل حسب نوع المادة النباتية وقيمة pH نتيجة اختزال أيونات الفضة Ag في المستخلص بعد إضافة محلول نترات الفضة AgNO₃ بنسبة حجمية (1 / 1) حجم/حجم. تم استخدام التحليل الطيفي للأشعة المرئية وفوق البنفسجية لإثبات وجود جسيمات الفضة النانوية، والذي أظهر ذروة امتصاص تتراوح بين (420-440 نانومتر) بسبب ظاهرة رنين البلازمون السطحي (SPR) للفضة النانوية، ولأجل توصيف جسيمات الفضة النانوية المنتجة من المزارع المختلفة استخدم المجهر الإلكتروني الماسح (SEM) الذي أظهر حجم الجسيمات النانوية التي تراوحت بين (18.341 - 40.932) نانومتر عند إنتاجها من مزارع الكالس و (17.261 - 29.13) نانومتر للجسيمات المحضرة من مزارع المعلقات المعلقة.

واستخدم التحليل الطيفي المشتت للطاقة (EDX) لمعرفة نسبة دقائق الفضة النانوية AgNPs المنتجة، حيث بلغت أعلى نسبة إنتاج 4.46% في مستخلصات المزارع المعلقة عند pH12، تليها مستخلصات الكالس، والتي وصلت إلى 4.12% عند pH12.

أثبتت جسيمات الفضة النانوية AgNPs المحضرة حيويًا فعاليتها في تثبيط نمو البكتيريا بنوعيهما الموجبة والسالبة لصبغة جرام، حيث تراوحت منطقة التثبيط (19-35 ملم) اعتمادًا على نوع المستخلص ودرجة الحامضية المطبقة على المستخلص، وكان لجسيمات الفضة النانوية المنتجة من مستخلصات مزارع المعلقات الخلوية أعلى قدرة تثبيطية، وتمثل هذه الفعالية إحدى أهم الخصائص الطبية لجسيمات الفضة النانوية.

الكلمات الدالة: حبة البركة، الانتاج الحيوي، جسيمات الفضة النانوية AgNps . كالس، المعلقات الخلوية، pH.